Upsala J Med Sci 98: 339–347, 1993

6.1.2.3 Prenatal Screening for Neural Tube Defects, Quality Specification for Maternal Serum Alphafetoprotein Analysis

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SUMMARY

The bimodal approach for quality specification in prenatal screening for neural tube defects has been used.

The method for assessing specifications has been the expenses for the society.

It has been shown that the cut-off values and analytical bias are of importance. It is recommended to use a reference serum for local calibration to keep the level within defined limits when changing to a new batch of reagents.

CLINICAL SITUATION

Second trimester maternal serum AFP (MS-AFP) analyses are used in screening for fetal malformations, especially neural tube defects (anencephaly and spina bifida) and abdominal wall defects. Increased levels of MS-AFP is found in nearly all cases of anencephaly, but only in 80-90% of open spina bifida cases. This is due to overlapping between the MS-AFP distribution curves for normal pregnancies and for spina bifida (SB) pregnancies. Therefore, both false negative and false positive results are seen. False negative results e.g. birth of a SB infant is found in one case out of 10 000-20 000 screening pregnancies whereas false positive results (elevated MS-AFP) are seen in 3-5% of all screening pregnancies. In these cases ultrasonography scanning (type 1 scanning) should be carried out in order to confirm gestational age and identify twin pregnancy. In less than 1% a malformation scanning (type 2 scanning) may also be carried out or an amniocentese with analyses of AFP and acetylcholinesterase for confirmation of the

screening result. These further measures cause major anxiety, are expensive, and amniocentesis is an invasive procedure which gives rise to abortions in about 1% of the cases.

MS-AFP concentrations are dependent of gestational age and the analyte concentration is normalized by conversion to multiples of the median analyte concentration for the gestational week (MoM). The number of fetuses are also of importance, since twin pregnancies having a two-fold AFP concentration compared to singletons.

MoM values greater than 2-2.5 MoM indicate an increased risk for SB. Expressing an actual measured value relative to a reference value determined at an earlier time makes the system vulnerable to bias whereas precision is of less importance; the CV_{At} beeing about 5% compared to a CV_{Bt} of about 45%.

METHOD

An immunofluorometric method (Delfia, Wallac, Turku, Finland) was used as a two-step incubation procedure according to the manufacturers instruction.

MODEL

The clinical situation is a typical bimodal problem.

EVALUATION OF QUALITY SPECIFICATIONS

The economic consequences of false positive and negative classified individuals have been used for evaluation of the model. The factors which influence such a model are the correctness of AFP - distribution curves for individuals who carry a normal fetus or a fetus with SB. The former is well defined while the latter is less well defined due to the few cases with SB.

The log Gaussian distribution curves for MS-AFP in normal pregnancies and in pregnancies with SB fetuses are shown in fig. 1.

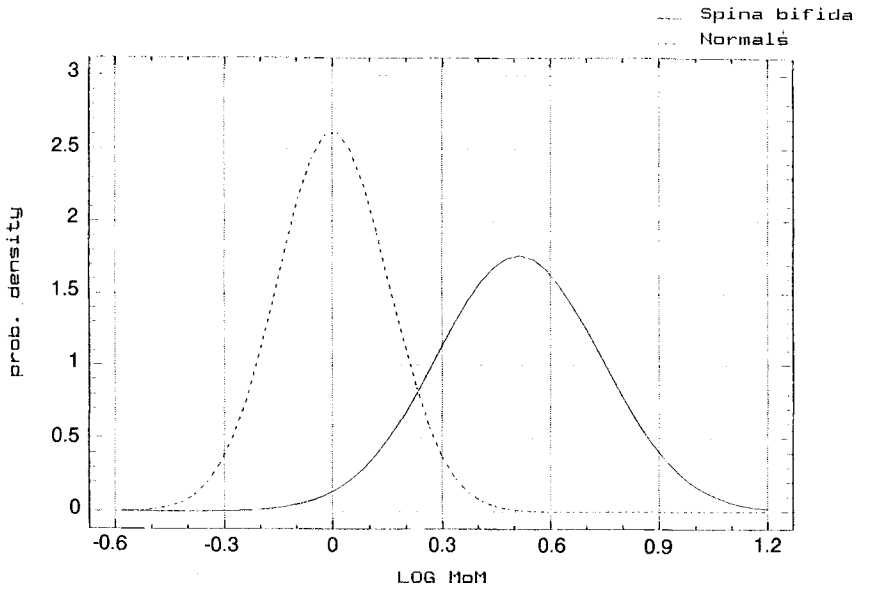

Figure 1. $Log_{(10)}$ Gaussian distribution for MS-AFP in normal pregnancies and pregnancies with SB fetuses.

By accumulating the areas under the curves SB from left to the right and normals from right to the left and plotting the areas on a probit scale two straight lines are formed and the percentage false positives and false negative can be read directly from the scale (fig. 2) (3).

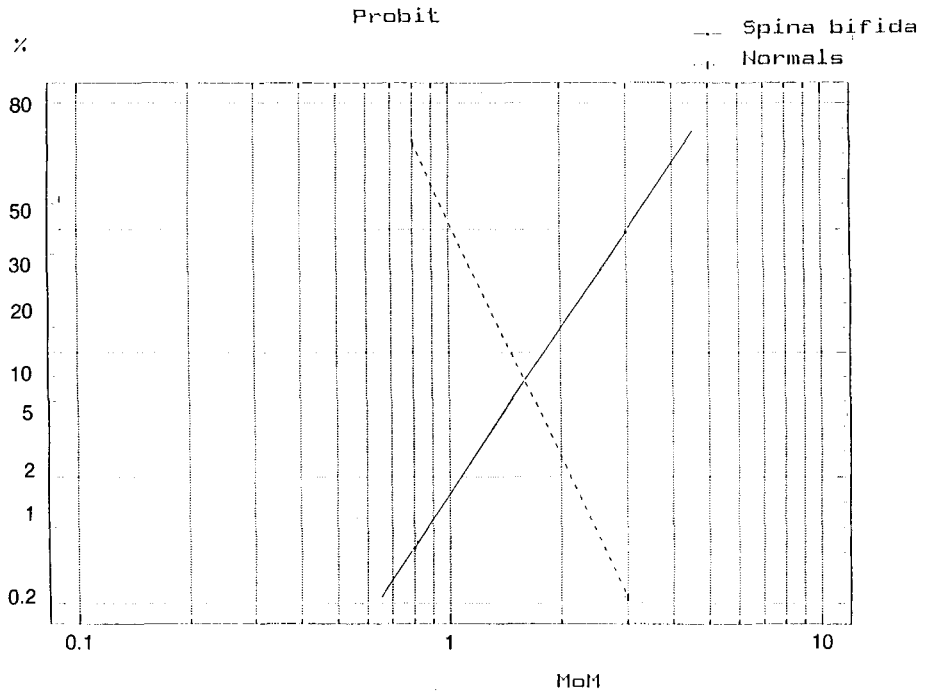

Figure 2. Probit transformation of MS-AFP distribution in normal pregnancies and pregnancies with SB fetuses.

There is no universal consensus of the MoM value which is considered positive, but a MoM value between 2 and 2.5 is normally taken as a cut-off value. The clinical consequences of a positive screening result is ultrasonography for detection of multiple pregnancies and for confirmation of gestational age or even a more demanding diagnostic ultrasonography for malformations. For about 1/4 of the women with increased MS-AFP it will be necessary to make an amniocentesis for the diagnostic amniotic fluid AFP and AChE analysis. It should be noted, however, that there is a clear tendency to replace the more expensive amniocentesis with a malformation scanning, because of better equipment and more skilled personal.

The economic consequenses of false positive and false negative results are based on a cost benefit report "Analysis of the economic consequenses of S-AFP screening and ultrasound screening in antenatal care" prepared by the ministry of the Interior in 1988 (5).

The figures used, table 1, are expenses in 1986 DKK and no attempts have been made to bring the figures up to date, hence the consequenses of an analytical bias are expressed as percentage changes in price.

Table 1.

POSSIBLE CONSEQUENCES OF A POSITIVE MS-AFP TEST

Ultrasonography	DKK.	92
1/2 hr medical advice	DKK.	50
Loss in productivity (4 x 74)	DKK.	296
Transportation	DKK.	50
	DKK.	**488**
Amniocentesis	DKK.	2580
1 hr medical advice	DKK.	100
Loss in productivity (4 x 74)	DKK.	296
Transportation	DKK.	50
	DKK.	**2946**
1 case of SB (over a period of 7 years)	**DKK.**	**303.000 /yr**

1986 prices

The number of false positive and false negative at different MoM levels can be converted to expenses based on the price of ultrasonography/amniocentesis and the expenses of the care of a case of SB respectively (fig. 3).

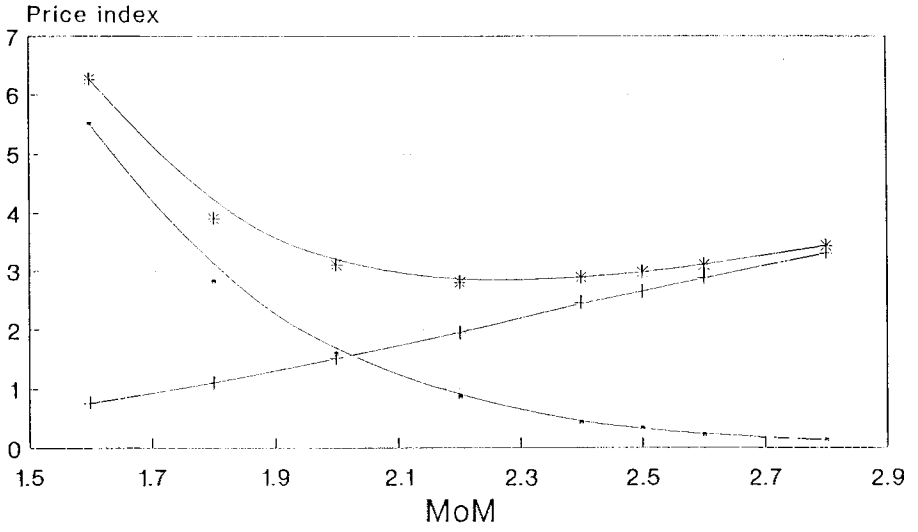

Figure 3. Relationship between expences and cut-off value in MoM.

FP FN Sum

The total expenses are dependent on the chosen cut-off value in MoM but are relative stable in the range of 2.1 to 2.5 MoM. At a given cut-off value a positive bias means that number of false positive and hence the expenses will rise. The influence of bias at different cut-off values is shown in fig. 4.

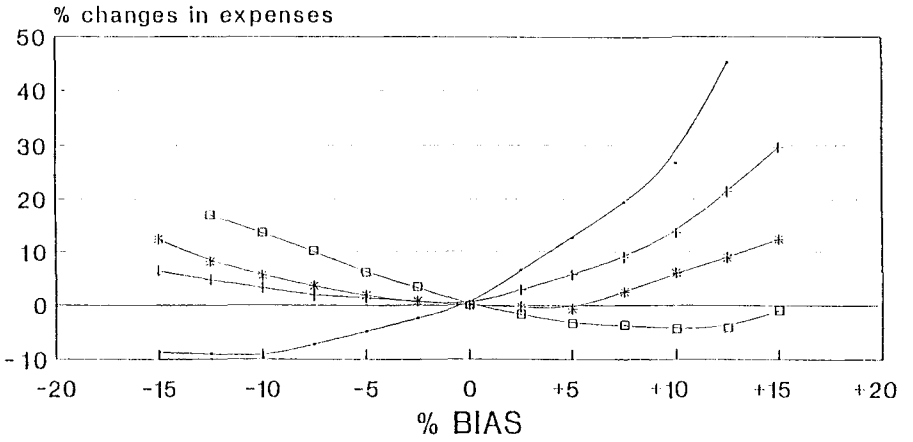

Figure 4. Relationship between expences and analytical bias at different cut-off values. Cut-off at: MoM = 2.0 —•— , 2.2 —+— , 2.3 —*—, and 2.5 —□—

A 5% increase of costs for misclassifications is considered acceptable for a cut-off of 2.3 MoM. This corresponds to an acceptable bias of ± 10%. According to the manufacturer the quality specifications for releasing a new batch are ± 7.5% based on analysis of several control sera. Those limits appear acceptable if a cut-off value of 2.3 MoM is used, since the changes in expenses will be less than 5%.

CONTROL SYSTEM

In practice, however, a greater difference in level between batches can be seen (fig. 5).

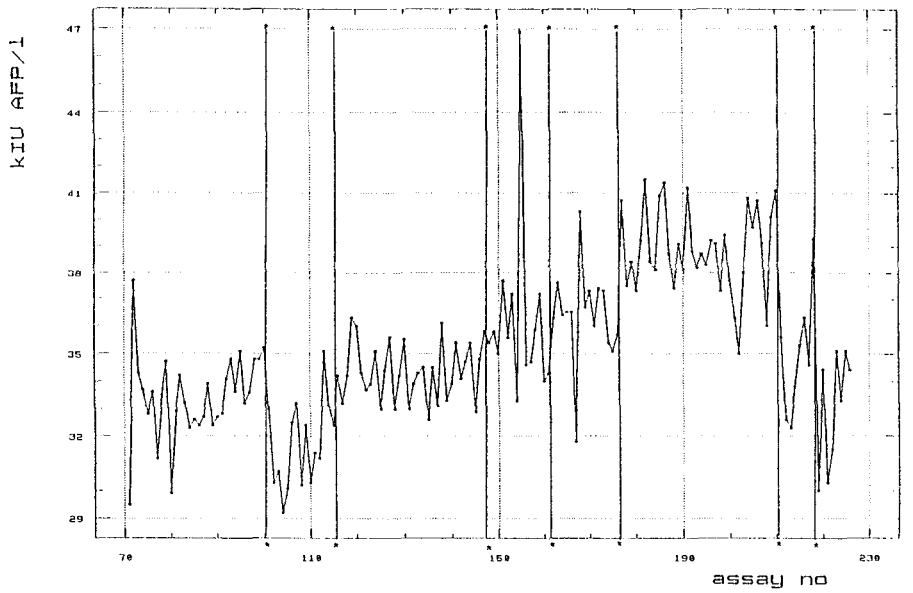

Figure 5. Mean of kIU AFP/l for control serum, 12 replicate in each assay *——* denote shift to new batch.

As AFP is a stable protein a serum pool from pregnant women can be used as a reference for internal calibration in order to keep the level within narrow limits when changing to a new batch. Further, to see if the limits chosen are correct the routine results of the patients samples can also be used (4), since nearly all the patients will be normal and therefore have a median MoM value of 1.0.

Each new reagent batch can be checked with the internal reference serum - and recalibrated if necessary.

The internal control system is based on another independent pool of sera from pregnant women, with the performance characteristics CV_{At} ~ 6.0%, CV_{Aw} ~ 4.0%, CV_{Ab} ~ 4.4%, and 12 replicants of this control are determined in each run.

$$CV_x = \sqrt{\frac{4.0^2}{12} + 4.4^2} = 4.5\%$$

Control rules:

Range rule: Range $\leq 4.0 \cdot 5 \sim 20\%$, $P_{fr} \sim 0.1\%$, $P_{ed} \sim 80\%$ for CV_{Aw} (max) $\sim 4.0 \cdot 2.5 \sim 10\%$

Mean rule: Limits: $\bar{\bar{X}} \pm 3 \times 4.5 \sim \bar{\bar{X}} \pm 13.5\%$. $P_{fr} \sim 0.3\%$, $P_{ed} \sim 50\%$ for bias $\sim \pm 13.5\%$, or $P_{ed} \sim 84\%$ for bias $\sim \pm 18\%$

The range rule is satisfying but the mean rule cannot fulfil the bias specifications of maximum bias $\sim \pm 10\%$. Therefore, results from four runs are combined in a moving average (of four runs with $CV_{MA} \sim \frac{4.5}{2} = 2.3\%$) which gives a delayed probability of detection of a persistent bias within the $\pm 10\%$.

The main problem of internal control is the between run component, and this must be reduced if a control system capable of guaranteeing the quality of each run within the limits of $\pm 10\%$ should be established.

DISCUSSION

With the model used it is shown that both the cut-off value and analytical bias are of importance. The performance of the analysis used with $CV_{At} \sim 6\%$, which is comparable to other kits for AFP analysis makes it difficult to design a control system which secure effectively against systematic error. The use of an economic model results in much more rigorous goals than models based on biological variation. The intra- and inter biological coefficients of variation amounts to 26.3% and 37.5% respectively (1). Using these figures and e.g. Cotlove's rule (2) would lead to a goal of 23% CV.

CONCLUSION

The economic consequences of maternal serum AFP analyses for fetal malformation screening based on a bimodal model have been calculated. It has been shown that the cut-off values chosen and analytical bias are of importance. It is recommended to use a reference serum for local calibration to keep the level within defined limits when changing to a new batch of reagents. Effective quality control rules with low probability for false rejection are difficult to construct due to the high between run variation.

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